

AMENDMENTS TO THE SPECIFICATION

At page 7, please replace the fourth full paragraph (lines 13-20) with the following paragraph:

Figure 4 is an alignment of the amino acid sequence of 17 bacterial LuxS polypeptides with the predicted translated amino acid sequence of *B. anthracis* ORF BA5464 (SEQ ID NO: 2). The 17 bacterial LuxS polypeptides that are shown are: *E. coli* (SEQ ID NO: 3), *S. typhimurium* (SEQ ID NO: 19), *Y. pestis* (SEQ ID NO: 6), *V. cholerae* (SEQ ID NO: 5), *V. harveyi* (SEQ ID NO: 4), *H. influenzae* (SEQ ID NO: 7), *N. meningitidis* (SEQ ID NO: 8), *C. jejuni* (SEQ ID NO: 9), *B. subtilis* (SEQ ID NO: 13), *B. halodurans* (SEQ ID NO: 14), *S. aureus* (SEQ ID NO: 11), *L. monocytogenes* (SEQ ID NO: 15), *H. pylori* (SEQ ID NO: 12), *E. faecalis* (SEQ ID NO: 10), *C. botulinum* (SEQ ID NO: 16), *S. pyogenes* (SEQ ID NO: 17), and *S. pneumoniae* (SEQ ID NO: 18). Amino acid residues are indicated by standard one letter abbreviation. Black shading indicates amino acids that are conserved in all (18 of 18) sequences (100% conservation across bacterial species), dark grey shading indicates amino acids conserved in ≥ 14 of 18 sequences ($\geq 77\%$ conservation across bacterial species), and light grey shading indicates amino acids conserved ≥ 11 of 18 sequences ($\geq 61\%$ conservation across bacterial species). Asterisks denote residues predicted to be involved in LuxS enzymatic activity (Hilgers and Ludwig. Proc. Natl. Acad. Sci. USA. 2001;98:11169-11174).

At page 30, please replace the third full paragraph (lines 23-30) with the following paragraph:

For oral formulations, the location of release may be the stomach, the small intestine (the duodenum, the jejunum, or the ileum), or the large intestine. One skilled in the art has available formulations which will not dissolve in the stomach, yet will release the material in the duodenum or elsewhere in the intestine, *e.g.*, by the use of an enteric coating. Examples of the more common inert ingredients that are used as enteric coatings are cellulose acetate trimellitate (CAT), hydroxypropylmethylcellulose phthalate (HPMCP), HPMCP 50, HPMCP 55, polyvinyl acetate phthalate (PVAP), Eudragit[®] L30D polymer, Aquateric[®] aqueous enteric coating, cellulose acetate phthalate (CAP), Eudragit[®] L polymer, Eudragit[®] S polymer, and Shellac. These coatings may be used as mixed films.

At page 31, please replace the second full paragraph (lines 9-13) with the following paragraph:

One may dilute or increase the volume of the therapeutic agent with an inert material. These diluents could include carbohydrates, especially mannitol, α -lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts may be also be used as fillers including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo[®] lactose, Emdex[®] diluent, STA-Rx 1500[®] diluent, Emcompress[®] diluent and Avicell[™] diluent.

At page 31, please replace the third full paragraph (lines 14-21) with the following paragraph:

Disintegrants may be included in the formulation of the therapeutic agent into a solid dosage form. Materials used as disintegrates include but are not limited to starch, including the commercial disintegrant based on starch, Explotab[®] disintegrant, Sodium starch glycolate, Amberlite[™] disintegrant, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. The disintegrants may also be insoluble cationic exchange resins. Powdered gums may be used as disintegrants and as binders. and can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

Please replace the paragraph bridging pages 31 and 32 with the following paragraph:

An antifrictional agent may be included in the formulation to prevent sticking during the formulation process. Lubricants may be used as a layer between the peptide (or derivative) and the die wall, and these can include but are not limited to; stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants may also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, Carbowax[™] 4000 and 6000 polyethylene glycol.

At page 32, please replace the third full paragraph (lines 15-21) with the following paragraph:

Controlled release oral formulations may be used in practicing the present invention. The therapeutic agent could be incorporated into an inert matrix which permits release by either diffusion or leaching mechanisms, *e.g.*, gums. Slowly degenerating matrices may also be incorporated into the formulation. Some enteric coatings also have a delayed release effect. Another form of a controlled release is by a method based on the Oros[®] therapeutic system (Alza Corp.), *i.e.* the therapeutic agent is enclosed in a semipermeable membrane which allows water to enter and push agent out through a single small opening due to osmotic effects.

At page 34, please replace the first full paragraph (lines 12-23) with the following paragraph:

Contemplated for use in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of therapeutic products, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art. Some specific examples of commercially available devices suitable for the practice of this invention are the Ultravent[™] nebulizer (Mallinckrodt Inc., St. Louis, MO); the Acorn II[®] nebulizer (Marquest Medical Products, Englewood, CO); the Ventolin[®] metered dose inhaler (Glaxo Inc., Research Triangle Park, NC); and the Spinhaler[®] powder inhaler (Fisons Corp., Bedford, MA). All such devices require the use of formulations suitable for the dispensing of the therapeutic agent. Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in addition to the usual diluents, adjuvants, surfactants and/or carriers useful in therapy. Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated.

At page 38, please replace the first full paragraph (lines 8-17) with the following paragraph:

The unfinished genomic sequence of the Ames strain of *B. anthracis* has been made publicly available, and partially annotated, by The Institute for Genomic Research (www.TIGR.org). Using

the 471-bp *B. subtilis luxS* gene sequence (via Genbank Accession # CAB15045.1; SEQ ID NO: 20, Figure 15) as a template, the partially annotated *B. anthracis* genome was subjected to BLASTN search under standard parameters using the search interface and algorithm available at <http://tigrblast.tigr.org/ufmg/>. This search identified a 474-bp predicted open reading frame (ORF), BA5464, with 72% sequence identity to *luxS* (also known as *ytjB*) from *B. subtilis* (see Figure 3). The nucleotide sequence of this putative *B. anthracis luxS* gene (SEQ ID NO: 1) is depicted in Figure 1. The transcriptional orientations of the flanking predicted ORFs BA5465 and BA5463 indicate that BA5464 is in a monocistronic operon (Figure 3).

At page 39, please replace the first full paragraph (lines 7-19) with the following paragraph:

An alignment of the predicted translated sequence of *B. anthracis* predicted ORF BA5464 with the amino acid sequence of LuxS polypeptide amino acid sequences from 17 other bacteria, suggests that BA5464 encodes a functional LuxS polypeptide (Figure 4). This alignment was generated from LuxS protein sequences retrieved from the N.C.B.I. and T.I.G.R. databases using the ClustalW algorithm (Thompson, Higgins and Gibson. Nucleic Acids Res. 1994;22:4673-4680) available, for example, at <http://www.ebi.ac.uk/clustalw/>. Although size variation exists between the LuxS polypeptides, conserved regions essential for function across prokaryotic genera have been defined (Miller and Bassler. Annu. Rev. Microbiol. 2001;55:165-169). Alignment of protein sequences from 17 known bacterial LuxS polypeptides with the predicted amino acid sequence of ORF BA5464 reveals a number of conserved amino acids, including those hypothesized to be essential for LuxS enzymatic activity (Hilgers and Ludwig. Proc. Natl. Acad. Sci. USA. 2001;98:11169-11174). Conservation of the amino acid residues thought to be essential for enzymatic activity of LuxS suggests that *B. anthracis* ORF BA6454 encodes a functional LuxS polypeptide with function.

At page 41, please replace the first full paragraph (lines 3-6) with the following paragraph:

E. coli strains DH5 α [®] strain (Promega) and SCS110 (Stratagene) were routinely grown at 37°C with aeration in Luria-Bertani broth (LB: 10g bacto-tryptone (Difco), 5g yeast extract (Difco), and 5g NaCl). Ampicillin (50 μ g/ml) was added to LB broth in cases for selection of *E. coli* strains harboring recombinant plasmids.

At page 41, please replace Table 1 with the following Table:

TABLE 1. Bacterial strains used in this study

Strain	Relevant characteristics	Source
<i>E. coli</i> DH5 α [®] strain	F-,080dlacZDM15, D(lacZYA-argF)U169, deoR, recA1 endA1, hsdR17(rk-, mk+), phoA, supE44, 1-, thi-1, gyrA96, relA1	Promega
<i>E. coli</i> SCS110	rpsL (Str ^R) thr leu thi-1 lacY galK galT ara tonA tsx dam dcm sup44 D(lac-proAB) [F' traD36 proAB lacIqZDM15]	Stratagene
<i>B. anthracis</i> 34F ₂	pXO1 ⁺ /pXO2 ⁻	Colorado Serum Co.
<i>V. harveyi</i> BB170	Sensor AI-1 ⁻ /Sensor AI-2 ⁺	Quorex, Inc.

Please replace the paragraph bridging pages 41 and 42 with the following paragraph:

V. harveyi bioluminescence assays. *V. harveyi* bioluminescence assays were performed essentially as previously described (Surette and Bassler. Proc. Natl. Acad. Sci. USA 1998;95:7046-7050). Briefly, *V. harveyi* strain BB170 was grown at 30°C in AB medium with aeration for 16h, cultures were diluted 1:10,000 in fresh AB broth, and then CFM from the bacterial cells to be tested was added to the culture (10% by volume CFM final concentration IN culture). Aliquots of 1.0 ml were taken 2 and 4 hours after CFM was added, and bioluminescence measured using a luminometer (Lumat[®] LB9507 from EG&G Berthold).

At page 43, please replace the second full paragraph (lines 15-23) with the following paragraph:

Construction of pMJ501. Chromosomal DNA of *B. anthracis* strain 34F₂ was purified using the Wizard[®] Genomic DNA Purification Kit according to manufacturer's instructions (Promega, Madison, WI). Purified genomic DNA was then used as template for PCR amplification of ORF BA5464. The PCR primers used were designated BALuxSF1 (5'-ATG CCA TCA GTA GAA AGC TTT G-3'; SEQ ID NO: 21) and BALuxSR2 (5'-CCA AAT ACT TTC TCA AGT TCA TC-3'; SEQ ID NO: 22). PCR was performed under standard conditions using the following cycling parameters: 30 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 51°C, and extension for 1 min at 72°C. In the normal course of this reaction, the Taq polymerase adds single adenine nucleotides to the 3' ends of the PCR product, to generate 1 nucleotide 3' overhangs.

At page 43, please replace the third full paragraph (lines 24-27) with the following paragraph:

This amplified PCR product was ligated with linear pGEM[®]-T Easy vector as per manufacturer's instructions (Promega) to produce pMJ501. pGEM[®]-T easy vector is a linear vector with single nucleotide 3' thymine overhangs, enabling ligation to the amplified product without the need for restriction sites.

Please replace the paragraph bridging pages 44 and 45 with the following paragraph:

The *B. anthracis luxS* ORF BA5464 was amplified by PCR and cloned into the *E. coli* shuttle vector pGEM[®]-T Easy vector to create pMJ501, in which expression of ORF BA5464 is under the control of an IPTG-inducible promoter. Cell free medium was prepared from high-density cultures of DH5 α [®]strain containing vector pMJ501 that had been induced with IPTG. The prepared CFM was then screened for the presence of synthesized AI-2 using the *V. harveyi* bioluminescence assay (Figure 6). As previously shown, compared to baseline luminescence seen with sterile CFM alone, CFM from high-density cultures of strain BB170 and *B. anthracis* 34F₂ induced substantial bioluminescence (Figure 6). As expected, no bioluminescence was induced by CFM from cultures of DH5 α [®]strain, or from DH5 α [®]strain containing pGEM-T Easy without an insert. In contrast, CFM from DH5 α [®]strain containing pMJ501 induced a high level of

bioluminescence, greater than that induced by CFM from the positive controls. Compared to the control *E. coli* DH5 α [®] strain CFMs, there was a 300-fold to a 1000-fold mean increase in induction of bioluminescence by pMJ501. This result confirms that expression of *B. anthracis* ORF BA5464 can complement the *E. coli* DH5 α *luxS* mutation and restore synthesis of AI-2, indicating that ORF BA5464 encodes a functional *B. anthracis* LuxS polypeptide.

At page 45, please replace the first full paragraph (lines 8-20) with the following paragraph:

A *B. anthracis* strain in which the *luxS* gene is mutated was constructed as follows (see Figure 7):

Chromosomal DNA of *B. anthracis* strain 34F₂ was purified using the Wizard[®] Genomic DNA Purification Kit according to manufacturer's instructions (Promega, Madison, WI). Purified genomic DNA was then used as template for PCR amplification of 1.18-kb and 989-bp genomic DNA fragments flanking the *B. anthracis luxS* gene (see Figure 7A). The 1.18 kb downstream fragment (F1/R1) was amplified using primers BALuxSKOF1 (5'-GAC TCA GTA ACA GAA CGT CGG-3'; SEQ ID NO: 23) and BALuxSKOR1 (5'-CGC AAT CTC TTA CAT AAG GTG-3'; SEQ ID NO: 24). The 989 bp upstream fragment (F2/R2) was amplified using primers BALuxSKOF2 (5'-CAC ATG TGG TCA AGC GAA G-3'; SEQ ID NO: 25), and BALuxSKOR2 (5'-GCC ACA TCA TAT CCA GTA TTC G-3'; SEQ ID NO: 26). PCR was performed under standard conditions using the following cycling parameters: 30 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 52°C, and extension for 1 min 30 sec at 72°C for 30 cycles.

At page 45, please replace the second full paragraph (lines 21-26) with the following paragraph:

The PCR products were purified using a Qiagen[®] PCR purification kit according to manufacturer's instructions (Qiagen). The purified fragments were then digested with *Hind*III, to generate fragments with a *Hind*III half site on one end and a 3' adenine overhang on the other end (the adenine was introduced as a natural consequence of Taq polymerase activity). The two *Hind*III

digested PCR fragments were then ligated to the linear pGEM[®]-T vector, which contains 5' thymine overhangs.

At page 50, please replace the fourth full paragraph (lines 21-26) with the following paragraph:

The overnight pre-incubation cultures were used to inoculate 50mL of fresh BHI medium, such that the new cultures had an optical density (OD₆₀₀) of 0.03 at the beginning of the culture period. The fresh BHI medium contained either 0µg/ml or 20µg/ml furanone 1 (final ethanol concentration = 0.12%). These cultures were grown at 37°C with aeration for 24hrs. This culture period represents the incubation period. The optical density (OD₆₀₀) of the incubation period cultures was measured at regular intervals. Optical densities at 600nm (OD₆₀₀) of the cultures were measured by reading 1-ml aliquots, using a Beckman DU[®]-7400 spectrophotometer.

At page 51, please replace the first full paragraph (lines 4-12) with the following paragraph:

A luxS gene mutation sensitizes B. anthracis to growth inhibition by furanone 1. *B. anthracis* strains 34F₂ and 34F₂Δ*luxS* were cultured overnight at 37°C with aeration in liquid BHI medium. These overnight cultures were used to inoculate 50mL of fresh BHI medium, such that the new cultures had an optical density (OD₆₀₀) of 0.03 at the beginning of the culture period. The fresh BHI medium contained either 0µg/ml or 20µg/ml furanone 1 (final ethanol concentration = 0.12%). Cells cultured with 0µg/ml furanone 1 served as the negative controls. These cultures were grown at 37°C with aeration for 30hrs and the optical density (OD₆₀₀) of the cultures measured at regular intervals. Optical densities at 600nm (OD₆₀₀) of the cultures were measured by reading 1-ml aliquots, using a Beckman DU[®]-7400 spectrophotometer.

Please replace the paragraph bridging pages 55 and 56 with the following paragraph:

B. anthracis 34F₂ cells were grown overnight in Brain Heart Infusion (BHI) broth at 37°C with aeration. Overnight cultures were diluted in fresh, sterile BHI, and cell densities were adjusted

to an OD₆₀₀ of approximately 0.03. Cultures were grown at 37°C with aeration. *B. anthracis* cells were treated 4-hrs post-inoculation with various concentrations of (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone ("furanone 1") dissolved in 95% ethanol such that the final concentration of furanone in the media was 0, 2.5, 10, or 20 µg/ml (final ethanol concentration = 0.12%). *B. anthracis* were also grown in the presence of EtOH to control for diluent. Cell densities were monitored by measuring OD₆₀₀. Cells were collected 15- and 30- minutes post-exposure to furanone 1. RNA was extracted, converted into cDNA, and labeled according to TIGR (The Institute for Genomic Research, Rockville, MD) protocols for the preparation of labeled cDNA for microarray assays. Briefly, cells were treated with RNA protect (Qiagen, Valencia, CA) and frozen to -70°C. RNA was extracted from samples using the Ambion bacterial RibopPure[®] kit according to Manufacturer's standard protocols (Ambion, Austin, TX). 2-µg of RNA was converted to cDNA and coupled to the fluorescent dyes cy3 and cyc-5. Labeled cDNA was hybridized to array slides containing the full genome of *B. anthracis*. Slides were scanned and spots analyzed by utilizing TIGR Spotfinder software (The Institute for Genomic Research, Rockville, MD). The array data was normalized for global intensity with TIGR MIDA (Microarray Data Analysis system) software. Genes were identified utilizing the TIGR TMEV (MultiExperiment Viewer) software. Genes that were considered significantly down- or up-regulated after furanone treatment had a log₂ ratio ≥1.5 after normalization.